RECENT ADVANCES IN OUR KNOWLEDGE OF THE Rh FACTOR*

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It seems appropriate to begin with a general survey of the problems which knowledge of the Rh factor has helped to clarify. As usual in scientific work, the problems become more complex as further insight is obtained, but without doubt the present state of knowledge is an immense advance over the conditions prevailing ten years ago, when Darrow (1938) so fully reviewed what was then known about the aetiology of haemolytic disease of the newborn. First, I should like to say that the term "Rh factor" as originally used seems unsuitable for the designation of all the antigens now known to exist in association with it in man, and I think it better to use the term "Rh blood group" to cover this field. The term "Rh factor" might then be used to distinguish the original component common to man and the rhesus monkey. It seems rather inappropriate to designate as Rh factors red cell antigens which have not been demonstrated in the cells of rhesus monkeys.

Discovery of the Rh Blood Group and its Clinical Significance

We owe our fundamental knowledge of the original Rh factor to the brilliant work of Landsteiner and Wiener (1941), who sought to extend knowledge of the complexities of agglutinin M in man and other primates by studying the reactions of human and animal bloods with anti-rhesus sera prepared by the immunization of rabbits or guinea-pigs by the washed red cells of rhesus monkeys. The sera thus prepared were found to cause agglutination of the red cells of 85% of white persons, who were therefore designated rhesus-positive or Rh-positive for short, the remaining 15% being called Rh-negative. This startling discovery was soon followed by the significant announcement that the newly discovered antigenic properties of human red cells were the basis of intragroup transfusion reactions as the result of iso-immunization of Rh-negative recipients by previous transfusion of Rh-positive blood (Wiener and Peters, 1940).

Within a remarkably short space of time a further pronouncement of major clinical importance was made. Levine announced that the dreaded intragroup transfusion reactions of women in the puerperium were also the result of iso-immunization of Rh-negative women, and drew attention to the almost invariable association of such reactions to a first transfusion with death of the foetus or the occurrence of icterus gravis neonatorum. Levine and his co-workers (1941) brought forward irrefutable evidence that not only the liability of the mother to intragroup transfusion reactions but also the disease and death of the foetus

were attributable to the iso-immunization of the mother by Rh antigens inherited by the foetus from an Rh-positive father. Somehow the antigen which the mother lacked had gained entrance to her circulation and had brought about iso-immunization with all its attendant troubles. These are the fundamental clinical facts about Rh incompatibility. Whatever advances have since been made or may be made in future, nothing can dim the brilliance of the achievements of our American colleagues, especially Wiener and Levine, in making these fundamental and fruitful observations.

Opportunities for iso-immunization of the pregnant woman by a foetal red cell antigen which she herself lacks is present in very many pregnancies—nearly 40% within the Rh group alone, according to Stancu, Clark, and Snyder (1947), but fortunately iso-immunization takes place only rarely. Clearly some red cell antigens are more effective immunizing agents than others, and also some women appear to be more readily immunized than others. Although the foetus has an A or B antigen which the mother lacks in about one-third of all pregnancies in this country, the ABO antigens only very rarely give rise to haemolytic disease of the foetus. None of the explanations of this freedom from ill effects in heterospecific pregnancy is wholly satisfactory, but clearly some potent protective mechanism must exist. There is also evidence that this protection is partially effective in preventing haemolytic disease due to coincident Rh incompatibility, for the incidence in heterospecific pregnancy is only about one-half of that found where the ABO groups of mother and foetus are compatible (Levine, 1943; Race, 1944; Wiener, 1945; Cappell, 1946). Transfusion of Rh-positive blood to an Rh-negative female before the first pregnancy has a very adverse effect and greatly increases the probability of haemolytic disease in the offspring, even if the transfusion has been given many years previously.

During the past year the Glasgow and West of Scotland Blood Transfusion Service under Dr. J. Wallace and Mr. Milne, to whom I am indebted for these figures, has examined the blood of approximately 25,000 women from the antenatal clinics of the area; among these were 4,540 Rh-negative women, 56 of whom were proved to have Rh antibodies, and in addition two Rh-positive women were found to be immunized and to have anti-E agglutinins of low titre. No fewer than 28 of the 56 Rh-negative mothers belonged to Group A and only 18 to Group O, a striking reversal of the A/O ratio for Scotland (Cappell, 1947b). Twenty-five of the fathers were examined and an equally striking disproportion in the opposite direction was found, only five out of 25 being of Group A and 15 out of 25 of Group O. These figures are consistent with previous findings that heterospecific pregnancy appears to confer some degree of protection against Rh sensitization.

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Varieties of Haemolytic Disease

In the absence of previous transfusion with Rh-positive blood iso-immunization of the Rh-negative mother rarely occurs during the first pregnancy to an extent sufficient to produce haemolytic disease in the first-born, but the second or subsequent Rh-positive offspring may be affected. Contrary to expectation statistical analysis shows that in affected families the first recorded manifestation of the disease is usually one of the severer forms. When the haemolytic disease occurs in the second pregnancy it is more likely to take the form of icterus gravis or hydrops than congenital haemolytic anaemia, which is seen more often when the first affected child comes later in the family—e.g. the fifth or sixth. If icterus gravis appears first, it is likely to be more severe in the next affected child or it may be followed by hydrops. Only rarely, however, do the harmful effects begin with mild congenital anaemia and progress with increasing severity in successive pregnancies through icterus gravis to hydrops foetalis. This early occurrence of the severer forms of the disease seems to indicate a notably greater susceptibility to immunization on the part of some women; thus cases with an early history of hydrops rarely show strong agglutinating antibodies but have usually developed blocking antibodies instead. Others may show strong agglutinating antibodies for some years, but in subsequent pregnancies blocking antibodies may appear (Cappell, 1947a, Case 4). Although we cannot predict with certainty the type of foetal disease from the type of antibody present, there is no doubt that the presence of blocking antibodies in high titre is a bad prognostic sign and is likely to be associated with premature death of the foetus or hydrops. In my own series of frank haemolytic disease cases with incomplete antibodies were about twice as numerous as cases with only agglutinins if we discount those in which agglutination occurred only in the first or second tube, where the protein concentration was high enough to lead to agglutinative effects with blocking antibodies (Wiener's conglutination reaction). Many of these earlier cases were studied before blocking antibodies were known, and their exact nature cannot now be ascertained. Sacks, Kuhns, and Jahn (1947) found among 12,140 unselected antenatal cases 96 Rh-negative sensitized mothers, of whom 65 had blocking antibodies alone, 23 had agglutinins alone, and eight had both types of antibody.

In Glasgow, however, among the above 56 Rh-negative sensitized women encountered in routine antenatal studies 33 showed anti-Rh agglutinins in a titre of 1 in 4 or more, while 23 showed blocking antibodies or a mixture of both types. The number of Rh-negative women showing sensitization is small, only about 1 in 80, but the antenatal clinics have an excess of primigravidae, who would be expected to produce infants free from haemolytic disease. Dr. Sandison, director of the East of Scotland Blood Transfusion Service, Dundee, has kindly supplied me with her recent figures showing that among 35 cases 23 had agglutinins of a titre greater than 1 in 4 and 12 had only blocking antibodies.

The Antigenic Structure of the Rh Group

The Rh group consists of eight principal Rh types, but there is evidence that variants of these types occur. Four of the principal Rh types are Rh-positive and four are Rh-negative. These types have been recognized by the reactions of the red cells with four different antisera, from which it is clear that each Rh type consists of three elementary antigens selected from three allelomorphous pairs. The true complexity of Rh antigenic structure was first recognized by the brilliant analysis and synthesis of R. A. Fisher, who recognized that the reactions of red cells with two of the four antisera were antithetical and supposed

that they distinguished allelomorphous antigens, which he called C and c. Since the reactions of the other two sera were not antithetical he supposed that they too reacted with elementary antigens and that each had an allele; these he named D or d, and E or e. Each Rh type was thus seen to be composed of three elementary antigens determined by three closely linked genes on the Rh chromosomes. The Rh types may thus be represented either by Wiener's shorthand notation such as R, R₁, R₂, R₃, r', r'', r₁, and r or by Fisher's more precise terminology using the CDE notation. As a result of the gradual advance in knowledge Wiener has had to admit that the Rh types consist of three elementary antigens, but he prefers to use a somewhat cumbersome terminology in which the c d e antigens are designated hr' Hr, hr'', hr' Hr₁, hr'', hr' Hr₂, hr'', hr' Hr₃, hr'', hr' Hr₄, hr'', hr' Hr₅, hr'', hr' Hr₆, hr'', hr' Hr₇, hr'', hr' Hr₈, hr'', hr' Hr₉, hr'', hr' Hr₁₀, hr'', hr' Hr₁₁, hr'', hr' Hr₁₂, hr'', hr' Hr₁₃, hr'', hr' Hr₁₄, hr'', hr' Hr₁₅, hr'', hr' Hr₁₆, hr'', hr' Hr₁₇, hr'', hr' Hr₁₈, hr'', hr' Hr₁₉, hr'', hr' Hr₂₀, hr'', hr' Hr₂₁, hr'', hr' Hr₂₂, hr'', hr' Hr₂₃, hr'', hr' Hr₂₄, hr'', hr' Hr₂₅, hr'', hr' Hr₂₆, hr'', hr' Hr₂₇, hr'', hr' Hr₂₈, hr'', hr' Hr₂₉, hr'', hr' Hr₃₀, hr'', hr' Hr₃₁, hr'', hr' Hr₃₂, hr'', hr' Hr₃₃, hr'', hr' Hr₃₄, hr'', hr' Hr₃₅, hr'', hr' Hr₃₆, hr'', hr' Hr₃₇, hr'', hr' Hr₃₈, hr'', hr' Hr₃₉, hr'', hr' Hr₄₀, hr'', hr' Hr₄₁, hr'', hr' Hr₄₂, hr'', hr' Hr₄₃, hr'', hr' Hr₄₄, hr'', hr' Hr₄₅, hr'', hr' Hr₄₆, hr'', hr' Hr₄₇, hr'', hr' Hr₄₈, hr'', 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variant of c—namely, c^V . The state of affairs at the D locus is similar. Stratton (1946) first recognized a different type of D antigen— D^U —by the observation that the cells in question failed to react with certain anti-D sera which were powerful against most D-containing cells. Race and his co-workers (1948b) have extended these observations and have shown that there are more than two types of D. By titration methods they have found that anti-D sera can be arranged in a certain order of reactivity, some sera agglutinating nearly all D cells while others fail to react with certain samples which are nevertheless readily sensitized by blocking anti-D sera, and are thus presumed to contain an antigen of the D class. Dr. Butler and I have observed at least two variants of D, one of which we detected in the cells of three out of 15 West African negroes. Such variants of D are probably attributable to the so-called intermediate genes which Wiener has reported among American negroes.

The Inheritance of the Rh Group

The Rh, like other blood groups, is inherited on Mendelian principles, the genes being carried by a chromosome pair different from those carrying all the other known blood groups. There are therefore three classes of persons, the homozygous Rh-positives (RhRh), the heterozygous Rh-positives (Rhrh), and the homozygous Rh-negatives (rhrh). The prognosis in any family in which haemolytic disease has appeared depends largely on whether the father is *homozygous*, when all his subsequent children are likely to be affected, or *heterozygous*, when one-half of the offspring are likely to be Rh-negative and thus escape harm. There are a few recorded instances of the Rh-positive child of an immunized Rh-negative mother remaining unaffected by haemolytic disease, but in some of the published cases the genotyping of the child is insufficiently detailed to prove that antigen D was present, and some such children have been r' (Cde) or r'' (cdE) and thus wrongly reckoned as Rh-positive. The use of Rh antisera containing two agglutinins—anti-C+D and anti-D+E (Wiener's anti-Rh₁ and anti-Rh₂)—for testing the baby's cells can easily lead to the wrong classification of infants as Rh-positives when in fact the positive reaction is due merely to the presence of antigen C or E. In some families Rh antibodies may appear in the mother and may even cause the infant's cells to give a positive direct reaction with Coombs's test, yet the child may remain clinically well or show only a trace of jaundice or anaemia. It is thus difficult to say on serological grounds that a child is unaffected if in fact its cells are sensitized, but no doubt the clinicians would be disinclined to accept such mild effects as examples of haemolytic disease. A discrepancy between laboratory and clinical findings may thus appear, but it will often be found in such families that subsequent Rh-positive children are affected beyond any clinical doubt, and this phenomenon may be merely evidence of commencing sensitization.

Example 1.—The difference in the prognosis with a homozygous father as compared with a heterozygous father is shown in the following family history.

Two Rh-negative sisters (rhrh) married Rh-positive men related as uncle and nephew. Both became immunized by three Rh-positive pregnancies, and haemolytic disease appeared in the third Rh-positive child. One man was homozygous RhRh, with the result that the fourth, fifth, and sixth pregnancies resulted in stillbirths, whereas the other man was heterozygous Rhrh, and his family of seven consisted of two Rh-negative children, a normal Rh-positive child, a second healthy Rh-positive child, two Rh-negative children, but the seventh pregnancy was again Rh-positive and resulted in haemolytic disease.

Example 2.—The late development of iso-immunization is shown in the following case, for details of which I am indebted to Dr. Sandison.

Mrs. D., group A, Rh-negative, had a family of 17 children, 15 of whom are alive and well—four are known to be Rh-negative and eight are Rh-positive. Recently an 18th child was apparently normal at birth, but its cells were strongly sensitized to Coombs's direct test; jaundice did not develop, and the child remained well, but by the 20th day its haemoglobin had fallen to 73%. The mother's serum contained only blocking antibodies.

Example 3.—Late sensitization, perhaps induced by transfusion.

Mrs. P., group O, Rh-negative, had seven normal children but received a blood transfusion in 1940 on account of haemorrhage after the birth of the seventh child. Four years later an eighth pregnancy terminated in a full-term male child who developed icterus gravis but recovered after transfusion of Rh-negative blood. The mother's serum contained blocking antibodies.

Example 4.—This family was important in our early work, as it was our first indication that there were different kinds of anti-Rh sera and that the Rh factor was more complex than had been realized; it also illustrates that Rh-negative women of genotype Cde/cde may become immunized against D.

Mrs. H., after losing her second and third children from icterus gravis, gave birth to a normal male child whose cells were agglutinated by some anti-Rh sera but not by others. Her serum contained a moderate amount of agglutinating anti-D, which reacted with the cells of her husband and first child but not with the cells of the fourth child. Later, the fifth and sixth children were also unaffected and proved to be Rh-negative cde/cde. The mother herself and the fourth child are of type Cde/cde ($r'r$) and thus might have been called Rh-positive by some observers.

The better understanding of the pathogenesis of haemolytic disease has not as yet yielded any notable advance in prophylaxis, and no effective means has been found either to prevent iso-immunization of Rh-negative women by Rh-positive fetuses or to render the effects of Rh antibodies less harmful. Kariher (1947) has reported that three sensitized women who had previously lost children from haemolytic disease were treated with repeated intramuscular injections of ethylene disulphonate in homoeopathic doses during a subsequent pregnancy. Their antibody titres fell markedly and each gave birth to a living Rh-positive child; two were unaffected and the third recovered under treatment. Confirmation of such interesting results is highly desirable.

Cerebral Damage

Hydrops foetalis is usually regarded as universally fatal, but I understand that Diamond has obtained a few survivors from the mild form after treatment by replacement transfusion. Icterus gravis neonatorum has a high mortality if untreated, but the use of Rh-negative blood for transfusion of the newborn infant has greatly improved the prognosis. Some very experienced workers, however, have expressed doubts about the value of treatment on account of the risk of cerebral damage (nuclear jaundice) and the chances of prolonging the lives of infants who are hopelessly crippled mentally or physically. I have thought it worth while therefore to report the late results of cases treated by Dr. McFarlane and me before 1945 and of others subsequently treated by Dr. Sandison, to whom I am indebted also for the results of the follow-up.

We have been able to re-examine 30 infants treated by transfusion of Rh-negative blood immediately after birth or mainly within the first 12 hours; all are normally developed physically and mentally, and not a single child shows evidence of backwardness. In comparison I am indebted to my colleagues of the Royal Hospital for Sick

Children, Glasgow, and particularly to Dr. R. R. Gordon, for the information that in a follow-up of a larger series of cases of icterus gravis admitted to that institution between 1934 and 1944 and treated mainly at a later stage by transfusion of blood not selected for Rh factor about 12% showed some residual nervous sequelae, such as general backwardness, a figure in close agreement with those given by Parsons (1947). Dr. G. L. Montgomery, pathologist to the Royal Hospital for Sick Children, Glasgow, informs me that during the same period the incidence of nuclear jaundice in fatal cases of icterus gravis was about 30%, a frequency notably higher than that of clinical signs of nervous involvement.

There is no doubt that nuclear jaundice may be unsuspected clinically in cases dying within the first few days. I am not convinced that the cerebral damage which results in nuclear jaundice occurs before birth, its incidence is so irregular and so poorly correlated with the amount or type of antibodies or the intensity of jaundice or anaemia that one is driven to speculate about other possible factors. In some cases observed personally I have been impressed with the difficulty in establishing respiration after birth, and perhaps anoxaemia in the immediately post-natal period may be a determining factor. While one must agree with Parsons (1946, 1947) that it is useless to prolong the life of the child known to be afflicted with nuclear jaundice, I am more optimistic than he, and I am hopeful that it may be possible at least to reduce the incidence of nervous sequelae by prompt and efficient treatment. Whether immediate replacement transfusion is the method of choice for this purpose, as Wallerstein (1946) and Wiener, Wexler, and Grundfast (1947) claim, can be decided only when a large series of cases has been treated by this technique.

In my opinion there is no evidence to suggest that Rh incompatibility between mother and child will itself lead to mental deficiency. General mental backwardness may result from damage to the cerebral cortex in those who have suffered from icterus gravis in the neonatal period, and if the basal nuclei have been severely damaged motor disturbances are very likely to follow in the survivors. The view of Yannet and Lieberman (1946) that mental deficiency occurs as the sole clinical manifestation of Rh incompatibility has not been borne out by subsequent studies (Scholl, Wheeler, and Snyder, 1947; Cappell, 1947a).

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PRELIMINARY NOTE ON INFLUENCE OF HETEROSPECIFIC IMMUNIZATION ON PRODUCTION OF Rh ANTIBODIES

BY

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In a previous paper (van Loghem, 1947) we reported the results of artificial immunization of male volunteer donors by frequent injections of small quantities of R' (Cde) and R'' (cdE) cells given intravenously in order to produce Rh agglutinins anti-C and anti-E. In two of the six volunteers the results were satisfactory. Rh agglutinins were formed in sufficient quantity to obtain useful Rh test sera. After the first experiments we were able gradually to extend the group of six male donors to 17. In general it takes a long time before the donors react with the formation of antibodies after repeated injections, and many of them do not react at all on the intravenous administration of these weak antigens (C, D, and E). Therefore we tried the influence of heterospecific immunization on the production of Rh antibodies.

It is a well-known fact that immunization with variola vaccine, typhoid vaccine, and many other vaccines may increase the titre of iso-agglutinins α or β .^{*} We tried this time to stimulate the production of Rh antibodies by simultaneous injections of two or three other antigens as well as the Rh antigens, where Rh antigens alone failed to act after prolonged administration. The vaccines used for this purpose were triplovaccine (1,000 million typhoid bacteria, 500 million paratyphoid A bacteria, and 500 million paratyphoid B bacteria per ml.) and tetravaccine (5,000 million cholera bacteria, 500 million typhoid bacteria, 500 million paratyphoid A bacteria, and 500 million paratyphoid B bacteria per ml.).

The donors selected for this purpose had already received many injections, varying from 15 to 42 in number, without producing Rh antibodies. Their sera were investigated at regular intervals by the saline and albumin methods.

Donor H. B.

H. B., aged 40, blood group A rr (cde/cde), had already received 42 injections, each of 1 ml. of a 25% red-cell suspension. The cells used for this purpose were type A R_r (cDe/cde), always derived from the same donor (see Table I). The injections were given intravenously mostly twice a week during a period of seven months, with three periods of rest varying from one month to eleven days.

When we started the administration of typhoid vaccine no Rh antibodies (early-immune or hyperimmune) were demonstrable in his serum. Triplovaccine was given three times intramuscularly at seven-day intervals in quantities of 0.5 ml., 1 ml., and 1 ml., but without results. After another nine injections with

^{*}Other arguments for the influence on the production of heterospecific antibodies by injection of heterospecific antigens were communicated: (1) by Hoet (1947), who suggested that in cases of erythroblastosis foetalis, in which the mother received previous to the Rh immunization by pregnancy injections of diphtheria, tetanus, or other vaccine, the mortality rate of the newborn increased; (2) by Pondman (1947), who reported that the production of antidiphtheritic serum in horses was stimulated by injections of a heterospecific antigen—tapioca; and (3) by our personal investigations (van Loghem and Spooander, 1948) which proved that ABO incompatibility in cases of Rh antagonism has an unfavourable influence on the clinical aspect of erythroblastosis foetalis.